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Isolation and Characterization of Soil Probiotic Bacteria in Cauvery River Basin and their Application in Aquaculture

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Research on probiotics for aquatic animals is growing along with the demand for environmentally friendly aquaculture. Probiotics have been defined as live microbial supplements that improve the health of humans and terrestrial animals. The micro biota in the digestive tract of fish and crustaceans is particularly dependent on the external environment due to the flow of water through the digestive tract. The need to increase disease resistance, growth of aquatic organisms and feed efficiency has led to the use of probiotics in aquaculture. In recent years, the use of probiotics in aquaculture has begun to increase. There is documented evidence that probiotics can improve

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nutrient digestibility, increase stress tolerance and promote reproduction. Unlike previous publications, this study focuses on the role of soil probiotic bacteria isolated from the Cauvery basin in Tiruchirapalli district. Isolated microorganisms - Nitrosococcus and Neisseria were tested for their special role in aquaculture, mainly in tilapia fish (Oreochromis sp.). This study also improves the bioremediation capacity of the isolated soil bacteria, which was demonstrated by testing the water quality parameters of the probiotic used. water environment Fish growth and mortality were also measured. It was clearly observed that the use of an isolated soil probiotic from a cave river basin promoted the longevity of tilapia fish. Thus, the cultivation and use of soil probiotic bacteria in aquaculture is a promising approach in modern science.

Keywords: Probiotics, aquaculture; cauvery river; Nitrosococcus; Neisseria; tilapia (Oreochromis sp.) bioremediation property; water quality; life sustainability.

1. INTRODUCTION

“Aquaculture is considered an important food security sector for a growing global population and has developed rapidly thanks to improved farming methods. The indiscriminate use of chemical additives and veterinary drugs as preventive and therapeutic measures has led to antimicrobial resistance of pathogenic bacteria and deterioration of environmental conditions” [1-3]. “As a result, serious losses due to the spread of diseases are recorded more and more. This significantly limits aquaculture production and trade and has a negative impact on the economic development of many countries. Several other methods have been considered to improve the quality and sustainability of aquaculture production. Among these methods, probiotics have been shown to play an important role in aquaculture” [4]. Despite the fact that probiotics offer a promising alternative to chemicals and antibiotics in aquatic animals [4] and contribute to the conservation of aquaculture species, the use of probiotics in aquaculture should be considered to avoid negative effects. result Since aquatic animals interact with different microorganisms in the animals and their habitat, the screening process for certain fish species is crucial to make them species-specific to obtain the desired results, with *In vitro* and *In vivo* experiments [5]. In addition, the choice of appropriate management methods leads to the creation of favorable conditions where probiotics can work well [6]. Probiotics have been widely used in routine water or nutritional supplements either as a single probiotic or in combination or even as a mixture with prebiotics or other immune response stimulants [7].

“Aquaculture is becoming one of the fastest growing and most promising industries providing animal protein and food security to a growing population. Due to its profitability, it also surpasses the agricultural sector. The expansion of the cultivated area and the intensification of farming practices lead to high animal densities [8]. The intensification of aquaculture practices has created stressful conditions for both aquatic animals and the environment. As a result, disease outbreaks are increasingly documented as one of the most significant threats to the aquaculture industry. Diseases and environmental degradation occur frequently and cause a serious situation. In recent decades, antibiotics have been used as a traditional strategy to control fish diseases and improve growth and feed conversion efficiency [9]. An alternative approach to fish and shrimp health management that has rapidly gained attention in the aquaculture industry is "probiotics," a microbial intervention approach to disease [10]. International Journal of Current Livelihood and food security for an ever-growing population. Consequently, aquaculture practices are expanded, improved and diversified to increase production [11]. This resulted in increased environmental and aquatic animal stress, ultimately resulting in disease outbreaks, reduced production, and threats to the sustainability of aquaculture [12]. In addition, they are considered a key focus for potential research into aquaculture disease control. In general, probiotic refers to Gram-positive bacteria, especially Lactobacillus sps, Bifidobacterium sps and Streptococcus, Bacillus sps, Lactococcus sps., Micrococcus sps., Carnobacterium sps., Enterococcus sps., Lactobacillus sps., Streptococcus sps., and Weissella sps. Microalgae (Tetraselmis) and yeasts (Debaryomyces), Phaffia and Saccharomyces” (Gatesoupe, 1999). There are several types of probiotics. This includes lactobacilli, bifidobacteria and some yeasts. Different probiotics have different effects [13]. This article summarizes the current knowledge on the use of probiotics in aquaculture. The world

of "probiotics" was invented by Parker (1974) and defined as "organisms and substances that influence the microbial balance of the intestine. Fuller (1989) changed the definition to "a live microbial food supplement that favorably affects the host animal by improving its gut microbial balance [14]. Probiotics are often referred to as "friendly", "beneficial", "good" or "beneficial" bacteria because they help keep the gut healthy [15]. More recently, probiotics have been defined as “live microorganisms” that, when administered in sufficient amounts, confer health benefits on the host (FAO/WHO, 2001). Types of Probiotics There are three types of probiotics mentioned below: Water Probiotics: These are labeled in two forms i) dry forms ii) liquid forms. Liquid forms give positive results in less time compared to dry and spore forms of bacteria due to their lower density (Nageswara and Babu 2006). They play an important role in improving the water quality of cultural ponds. Soil probiotics: Nitrobacter, Nitrosomonas and sulfur reducing bacteria clean the bottom of water ponds [16]. Nutritional / intestinal probiotics: lactic acid bacteria. Probiotics act as a microbiological dietary drug that promotes host health by reducing mucosal and systemic immunity and enhancing physiological and nutritional effects [17]. They increase the efficiency of feeding fish and shrimp by stimulating digestive enzymes and maintaining the balance of intestinal microbes, which improves nutrient uptake, utilization, and ultimately fish and shrimp survival and growth [18]. The Role of Probiotics Three types of probiotic bacteria can be applied directly to soil, culture pond water and also as a food additive. Various commercial probiotics are available in the market in different combinations and bacterial numbers. The use of probiotic bacteria has been reported to reduce mortality [19]. However, the number of cells in the probiotic administered with the feed plays an important role in the survival of the animals. The potential effect of a probiotic depends on the source of bacterial isolation and the method of use [20].

2. MATERIALS AND METHODS

2.1 Isolation and Characterization of the Soil Probiotic Bacteria

The soil sample is collected from Cauvery basin in Tiruchirapalli district. The sample was dried and then checked for the presence of microbes.

2.1.1 Chemicals and rezagents

Nutrient Agar Medium, Nutrient Broth was purchased from Himedia, India. Whatman No. 1 filter paper, gentamicin antibiotic solution, test samples, test tubes, beakers, Erlenmeyer flasks, alcohol lamp, double distilled water, Petri dishes, TrisHcl, phenol-chloroform, methyl red, Kovac's reagent, KOH, and alpha-naphthol. Soil sample calculation 0. 1 g of the test sample was dissolved in 10 ml of sterile double distilled water and incubated for 1 hour at room temperature. The filter supernatant was used in serial dilutions of 101-108. Dilutions of 102 and 106 were used to isolate bacteria.

2.1.2 Nutrient agar medium

The medium was prepared by dissolving peptone - 0.25 g, NaCl - 0.25 g, yeast extract - 0.1 g, beef broth - 0.05 g and agar powder - 0.875 g in commercially available AMegard nutrient medium in H50. of distilled water. The dissolved medium was autoclaved at 15 pounds at 121°C for 15 minutes. The autoclaved medium was mixed well and poured into 100 mm Petri dishes (25-30 ml per dish) while still molten. Dilutions 102 and 106 were plated on nutrient agar using the spread plate method and the plate was incubated at 37°C for 24 hours. After incubation, bacterial colonies were isolated and plated on a fresh plate.

2.1.3 Nutrient broth

Nutrient broth was prepared by dissolving peptone - 0.25 g, NaCl - 0.25 g, yeast extract - 0.1 g, beef broth - 0.05 g and agar powder - 0.875 g in commercially available Nutrien (5 AMegar). incl. distilled water. The medium was aliquoted as desired and sterilized in an autoclave at 15 pounds (121°C) for 15 minutes.

2.1.4 Screening and identification of microbes

The medium was prepared by dissolving commercially available Nutrient Agar Medium (HiMedia) in 50 ml of distilled water with peptone - 0.25 g, NaCl - 0.25 g, yeast extract - 0.1 g, beef broth - 0.05 g and agar powder - 0.875 g. The dissolved medium was autoclaved at 15 pounds at 121°C for 15 minutes. The autoclaved medium was mixed well and poured into 100 mm Petri dishes (25-30 ml per dish) while still molten. 102 were isolated from three different colonies and 106 were isolated from two different colonies isolated from dilution of the test sample products.

Five different colonies were drawn on one Petri dish.

2.1.5 Gram staining : procedure

A loop full of bacterial culture was placed on the slide. The slip smeared the flame. Slides were stained with crystal violet and held for 1 minute, and slides were washed with distilled water. Gram's iodine was added and incubated for 1 minute, then washed with distilled water. Decolorizer was added and kept for 1 minute and then saffron broth was added, washed with distilled water after one minute. Slides were viewed under a trinocular microscope, purple colors indicate gram-positive bacteria and pink indicates gram-negative organisms.

2.1.6 Suspension method

The suspension technique is an established method for studying living, unstained very small organisms. The traditional method uses a glass plate with a circular well in the middle, from which a drop of liquid containing "microorganisms" hangs from a lid. Well slides are expensive and covers are delicate so some students find it difficult to work with them. Here we offer an alternative technique that is easy to use in the classroom. An easily changeable key film cover, blu-tak and two microscope slides provide an affordable and practical option that allows students to view live cultures easily and efficiently. Algae and protozoa are large enough for students to successfully view with a standard school microscope. The study of such "hanging drops" can lead to useful discussions about the size, diversity, properties and importance of microorganisms, and to think about the differences between "plant" and "animal" cells. The purpose of this activity is to allow students to experience the magnificence of the micro world that can exist in a suspended drop and to observe some of the microorganisms in fresh water. By viewing a properly aligned hanging drop preparation of the recommended mixed algae using lenses that provide x 100, x 200, or x 400 magnification, students can see a variety of different sized (mostly unicellular) algae. Some of them are mobile and swim across the field of view with amazing speed. Others, such as desmids, which have three perfect planes of symmetry, show interesting and remarkable forms, as do diatoms, whose individual cells display an amazingly complex architecture. This method is used to study microorganisms in "purchased" preparations, pond water or "home cultures". We recommend mixed cultures of algae and protozoa purchased from Sciento. (We found these Science cultures to be consistently high in both diversity and number of organisms.) For algae, make sure the drop contains visible green material so that a variety of cells and non-cells can be observed. - motile organisms (although a drop of "clear" liquid may contain limited amounts of motile organisms such as Chlorella). When sampling protozoa from a vessel, it is important to select a portion of the solid material because protozoa are likely to feed there. All algae can perform photosynthesis. Therefore, this activity can lead to discussions about the importance of algae in carbon sequestration in rivers, lakes (or lakes), seas and oceans, and also about phytoplankton in the early stages of aquatic food webs. Diatoms are believed to be important ingredients in the formation of oil deposits.

2.2 Application in Aquaculture

2.2.1 Acclimatization of the experimental animal

The experimental animal chosen for this study was Tilapia fish (*Oreochromis mossambicus*). Tilapia weighing approximately 5-8 grams and averaging 8-15 cm in length were collected from a nearby pond in Allithurai, Tiruchirappalli and acclimatized to laboratory conditions for about 10 days in large plastic containers that were previously washed with potassium permanganate to loosen. walls of any microbial growth. Food was regularly supplied in pellets. Fish were well aerated and non-chlorinated tap water was used.

2.2.2 Chloride estimation

10 ml water samples were taken in an Erlenmeyer flask and 2 drops of 5% potassium chromate solution were added as an indicator. The resulting lemon yellow solution was titrated with silver nitrate solution taken from a buret. After each drop of silver nitrate was added, the contents of the Erlenmeyer flask were shaken vigorously to prevent spillage of the precipitate. The end point was the appearance of a permanent brick red color. The titration was repeated to obtain constant observation values. Different samples were evaluated and readings were calculated.

2.2.3 Estimation of nitrite

0.2 ml of EDTA solution was added to 10 ml sample, blank and standard solutions and mixed

well. 0.2 ml of sulfanilic acid was added and mixed thoroughly. After 10 minutes, 0.2 ml sodium acetate and 0.2 ml α-naphthylamine hydrochloride solution were added to all tubes and mixed thoroughly. After 10 minutes, the resulting color was compared visually and the optical density was read at 530 nm. The evaluation took place several times.

2.2.4 Nitrate estimation

1 ml samples, standard and blank samples were taken separately in a test tube. 0.5 mL of brucine sulfanilic acid solution was added to each tube and mixed well. 5 ml of sulfuric acid solution was added to the second set of test tubes. The contents of the first set of test tubes were added to each of the other sets containing sulfuric acid and mixed well. These tubes were kept in the dark for 10 minutes. OD was measured at 510 nm and the procedure was followed for all samples.

2.2.5 Estimation of dissolved oxygen

(**Modified Winkler's Method)**

The level of dissolved oxygen (DO) in nature and wastewater depends on the physical, chemical and biochemical functions of the water body. DO analysis is a key test to monitor water pollution and waste treatment processes. Two methods are available for DO analysis: the Winkler or iodometric method and its variations, and the electrometric method, which uses membrane electrodes.

Objective: to estimate the amount of DO in the given water samples.

Principle: Based on the addition of a divalent manganese solution and then a strong alkali to the sample in a glass-stoppered bottle. SO rapidly oxidizes an equivalent amount of dispersed divalent manganese hydroxide precipitate to a higher valence hydroxide. In the presence of iodide ions in an acidic solution, the oxidized manganese returns to the divalent state, releasing iodine corresponding to the original DO concentration. The iodine is then titrated with standard thiosulfate solution. The end point of the titration is detected visually with a starch indicator. Acid modification is used in most wastewaters, effluents and streams containing more than 50 µg NO2-N/L and up to 1 mg iron/L.

Reagents:

1. Manganese sulfate reagent: 480 g Mn SO4.4 H2O or 400 g Mn SO4.2 H2O or 364 Mn SO4. H2O was dissolved in distilled water, filtered and diluted to 1 L.
2. Alkaline iodide azide reagent: a. 500 g NaOH or 700 g KOH and 135 g NaI or 150 g KI were dissolved in distilled water and diluted to 1 liter. b. 10 g of NaN3 was dissolved in 40 ml of distilled water. c. Both solutions (a) and (b) were mixed together to give the alkaline iodide azide reagent.
3. Concentrated H2SO4
4. Starch indicator: 0.5 g of starch powder was mixed with distilled water to form a paste. This paste was poured into 100 ml of boiling water and then cooled.
5. Sodium thiosulphate standard solution (0.025 N): 6.205 g Na2S2O3. 5H2O was dissolved in distilled water (previously boiled and cooled) and diluted to 1 liter. It was standardized against a standard potassium dichromate solution. (Note - the equivalent weight of sodium thiosulfate pentahydrate is its molecular weight).
6. Standard potassium dichromate solution: (0.1 N) 4.904 g of K2Cr2O7 were dried at 103 °C for 2 h, dissolved in distilled water and diluted to 1000 mL.

Procedure:

1. Standardization of sodium thiosulphate solution: 40 ml of cold, freshly boiled distilled water were placed in a 250 ml Erlenmeyer flask, 1,200 g of iodate-free potassium iodide and 800 mg of pure sodium bicarbonate. . dissolved 2. 4 mL of concentrated HCl was slowly added while gently swirling the flask. 10.0 mL of 0.1 N K2Cr2O7 solution was added and the contents were mixed well. The sides of the bottle were washed with a small amount of distilled water. The flask was covered with a small watch glass and allowed to stand in the dark for 5 minutes to complete the reaction. The watch glass was washed and the solution was diluted with about 120 ml of boiled cold water. The released iodine was titrated with sodium thiosulfate while continuously stirring the liquid until the color changed to yellow-green. At this point, 2 ml of the starch solution was added and the titration was continued by dropwise addition of thiosulfate until 1 drop changed its color from greenish blue to pale green. From this titration, the exact normal thiosulphate was calculated. 2 ml of manganese sulfate solution and 2 ml of alkali-

iodazide reagent were added to the sample collected in the BOD bottle (the reagents were added well below the surface of the sample water). The bottle was sealed without air bubbles and mixed by inverting the bottle several times. The precipitate was allowed to settle to the bottom of the flask. The cap was carefully removed and 2 ml conc. H 2 SO 4 was added and mixed well by inverting the flask several times until complete dissolution. A 50 mL sample from the flask was pipetted into a clean Erlenmeyer flask and titrated with 0.025 N thiosulfate solution until it turned a pale straw color. At this point, a few drops of the starch solution were added and the titration was continued by dropwise addition of thiosulfate until the blue amount of distilled water disappeared. From this titration, the exact normal thiosulphate was calculated.

Calculation:

$$\text{Dissolved oxygen (mg/1)} = \frac{V_{THIO}\, x\, N_{THIO}\, 8\, x1000}{V_2\, [(V_1 - V)/\,(V_1)]}$$

Where,

V_1 = Total volume of bottle after placing the stopper
V_2 = Volume of the sample taken for titration
V = Total volume of $MnSO_4$ + Alkali iodide

2.2.6 Estimation of free Carbon dioxide:

50 ml of the water sample was taken in a conical flask. A few drops of Phenolphthalein was added as indicator. This solution was titrated against 0.01N sodium hydroxide. The end point is the appearance of pale permanent pink colour. The titration was repeated for constant concordant values and it was estimated for few samples. Dissolved CO_2 has marked effects on the properties of water, if forms a weak carbonic acid solution that changes the pH increases, alkalinity and hardness of water by dissolving minerals.

Principle:

Free CO_2 reacts with NaOH or Na_2CO_3 to form Na $(HCO_3)_2$, the completion of the reaction is indicated by the appearance of pink color in the presence with phenolphthalein indicator as pH of 8.3.

Reagents:

- **Std. NaOH solution (0.02 N):** Dissolve 200 ml of stock solution to 1000 ml with distilled water.
- **Stock NaOH solution:** Dissolved 4 g NaOH in 1000 ml distilled water.
- **Phenolphthalein indicator:** Dissolve 0.5 g of phenolphthalein powder in 50ml of 95% C_2H_2OH and add 50ml distilled water.

Procedure:

- Take 50 ml of sample in a conical flask.
- Add 4 to 5 drops of phenolphthalein indicator:
  If the sample is pink color does not appear titrate the sample against standard 0.02 N NaOH and until the pale pink color develop and remain for 30 sec.
- Note down the burette reading

Calculation:

$$\text{Free CO2 (mg/l)} = \frac{\text{V x N x 44 x 1000}}{\text{Volume of sample}}$$

V = Volume of 0.02
N NaOHN = Normality of NaOH

2.2.7 Determination of oxygen consumption and Ammonia excretion

The test was performed once a week, immediately before the next water change. To measure O2 and ammonia consumption, the sealed container method is followed (Brett, 1964). The first half of each respiratory system is filled with water. Catches from the experimental and control groups were randomly collected, weighed and placed in their respirators before feeding. The inlet to the breathing system was closed, filled with water, and the orifices and outlet closed. Allow the fish to acclimatize and leave for an hour. In other words, the oxygen and ammonia content of the water was determined by Winkler's method and ammonia estimation. After an hour, water samples are injected and analyzed for oxygen and ammonia. The difference in oxygen content between the first and second sample shows how much oxygen the fish consumed in one hour. Also, the difference in ammonia in the two samples caused the fish to produce ammonia within an hour. The test was performed three times on each system as needed. Mean values were taken and fish

oxygen consumption and ammonia rate were calculated in mg/fish/hour. From the oxygen consumption and ammonia excretion rate, the ammonia index (A.Q) was calculated using the formula.

$$\text{Ammonia Quotient (A. Q)} = \frac{\text{Amount of ammonia excreted}}{\text{Amount of oxygen consumed}}$$

2.2.8 Biological Oxygen Demand (BOD)

and to evaluate the BOD removal efficiency of that treatment system. This test measures the oxygen used during the selected incubation period for the biochemical degradation of organic matter and the oxygen used to oxidize organic substances such as sulfides and iron ions. It is also possible to measure the oxygen used to neutralize the low nitrogen, if it is blocked by the obstruction. The incubation and dilution method provides an estimate of BOD at pH between 6.5 and 7.5. BOD concentrations in most wastewaters exceed dissolved oxygen (DO) concentrations available in sample gas concentrations. It is therefore necessary to dilute the sample prior to incubation to ensure an adequate balance between oxygen demand and supply. Since bacterial growth requires nutrients such as nitrogen, phosphorus, and trace metals, these nutrients are added to the buffer dilution water to maintain the pH of cultured samples as long as possible during the bacterial incubation period. Therefore, 5 days was accepted as the standard incubation period. Method: The sample is placed in a closed bottle and incubated in the bottle under the specified conditions for the specified time. Dissolved oxygen is first measured, and after incubation, BOD is calculated as the difference between the initial DO and the final DO.

Reagents required:

a. Phosphate buffer solution

8.5gms KH_2 PO_4, 21.75gms K_2HPO_4, 33.4gms, Na_2 $HPO_4$7H2O and 1.7 NH_4 C1 were dissolved in about 500 ml distilled water and diluted to one litre.

b. Magnesium Sulphate Solution

22.5gms. $MgSO_4$. $7H_2O$ was dissolved in distilled water and diluted to 1 litre.

c. Calcium Chloride Solution

27.5gms. $CaC1_2$ was dissolved in distilled water and diluted to 1 litre.

d. Ferric Chloride Solution

0.25gms. $FeC1_3$. 6 $H_2O$was dissolved in distilled water and diluted to 1 litre.

e. Acid and alkali Solutions

1N acid and alkali solutions for neutralization of alkaline or acidic waste samples.

f. Sodium Sulphite Solution

1.575 gms. Sodium Sulphite was dissolved in distilled water and diluted to 1 litre.

Procedure:

Phosphate phosphates, MgSO4, CaCl2 and FeCl3 were mixed with the required volume of distilled water at 1 ml per liter and heated for 30 minutes. This is called dilution water. The sample was diluted appropriately using a standard dilution table with dilution water. Two sets of BOD flasks were filled with solutions at each dilution interval. One bottle was incubated with the label in a BOD incubator at 20 degrees Celsius for 5 days. Initial DO levels were estimated and recorded from different sets of bottles. After 5 days, the final DO is calculated and the BOD is calculated using the following formula.
BOD mg/1 = (Initial DO-Final DO) x Dilution factor(100 to 1000 times)

2.2.9 Chemical Oxygen Demand(COD)

Principle:

Chemical oxygen consumption is a measure of oxygen consumption when an oxidizable organic substance is oxidized by a strong oxidizer. Potassium dichromate in the presence of sulfuric acid is usually used as an oxidizing agent for COD determination. Samples were refluxed with potassium dichromate and sulfuric acid in the presence of mercuric sulfate to neutralize the effect of chlorides and silver sulfate as a catalyst. Organic matter is completely oxidized to carbon dioxide and water. The remaining excess of potassium dichromate is titrated with ferrous ammonium sulfate using ferroin as an indicator. The amount of potassium dichromate consumed

is proportional to the oxidizing agent present in the sample.

Reagents required:

Potassium dichromate solution

12.25g of dried A.R grade potassium dichromate was dissolved in double distilled water and was made upto 1000ml.

Ferrous Amonium Sulphate (0.1N)

39.2g of FAS was dissolved in distilled water along with the addition of 20ml of concentrated Sulphuric acid. It was made upto 1000ml in a standard flask.

Silver Sulphate Concentrated Sulphuric Acid mixture:

10g of Silver Sulphate was dissolved in 1000ml of conc. Suphuric acid.

Ferroin indicator:

1.485g of 1,10 phenonthroline and 0.695g of ferrous sulphate was dissolved in distilled water and made upto 100ml.

Mercuric Sulphate

Procedure:

20 mL of the sample was placed in a round bottom flask and a small amount of sodium platinum was added. Added anti-shock granules. 5 mL of the mixture of sulfuric acid and silver sulfate was added to the solution in the flask and mixed well to promote dissolution of the mercuric sulfate. 10 ml of potassium dichromate was added and then 25 ml of the mixture of sulfuric acid and silver sulfate was added. Hold the lower round candle in an ice water bath while adding this mixture. This is to prevent the release of fatty acids due to the high temperature. The RB flask was then connected to a condenser and incubated for 2 hours. After 2 hours, cool the bottle, add 80 ml of distilled water and mix well. It was titrated with FAS solution using a ferroin indicator. The result is a sudden change in color from blue-green to wine-red. At the same time, the blank was prepared in the same way using distilled water instead of the sample containing the same amount of substances.

Calculation:

$$\text{COD (mg/1)} = (\text{B} - \text{A}) \text{ x } \frac{\text{N}_{\text{FAS}}\ 8\text{x}1000}{\text{Volume of sample}}$$

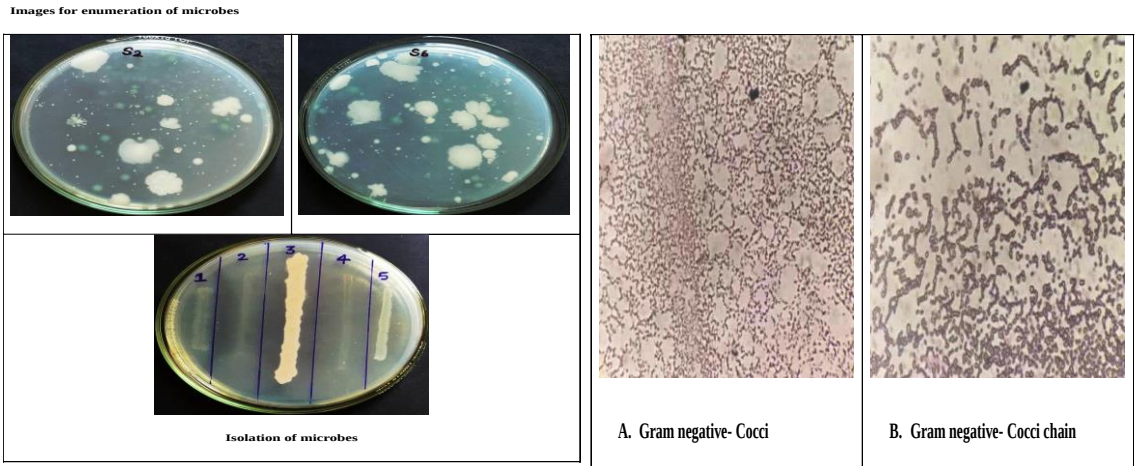
Where,

A = Volume of FAS for Sample ----- ml.
B = Volume of FAS for Blank ------ ml.

2.2.10 Growth and mortality

Growth and weight were recorded as percentages. During the experiment, mortality rates of fish after probiotic treatment were also observed and recorded.

3. RESULTS



S. No.	Name of the test sample concentration	Name of the Biochemical characterization						Identified bacteria
		Gram staining	Motility	Indole test	Methyl red test	Voges Proskauer	Simmons' test	
1.	A	Gram negative–Cocci	Motile	–	+	–	+	Nitrosococcus
2.	B	Gram negative–Cocci chain	Motile	–	+	–	+	Neisseria
3.	C	Gram positive Bacillus sp.	Motile	+	+	+	–	Lactobacillus acidophilus

Fig. 1. Acclimatization of the Tilapia fish and application of the probiotic supplementary food

Fig. 2. Physico – chemical parameters observed in the probiotic treated water

Table 1. Chloride Content

DAY	CONTROL			FISH TREATED WITH PROBIOTICS (10mg/l)		
	LOW	MEDIUM	HIGH	LOW	MEDIUM	HIGH
0	67.4 ± 2.80	67.4 ± 2.80	67.4 ± 2.80	67.4 ± 2.80	67.4 ± 2.80	67.4 ± 2.80
7	75.7 ± 1.60	80.4 ± 1.60	76.9 ± 1.60	82.8 ± 1.60	79.2 ± 1.60	79.2 ± 1.60
14	76.9 ± 1.60	73.3 ± 1.60	84.0 ± 1.60	80.4 ± 1.60	76.9 ± 1.60	80.4 ± 1.60
21	76.3 ± 1.60	79.8 ± 1.60	86.9 ± 1.60	83.4 ± 1.70	83.4 ± 1.70	79.8 ± 1.70

Table 2. Nitrite content recorded

DAY	CONTROL			FISH TREATED WITH PROBIOTICS (10mg/l)		
	LOW	MEDIUM	HIGH	LOW	MEDIUM	HIGH
0	0.073 ± 0.002	0.073 ± 0.002	0.073 ± 0.002	0.073 ± 0.002	0.073 ± 0.002	0.073 ± 0.002
7	0.042 ± 0.002	0.40 ± 0.004	0.042 ± 0.002	0.030 ± 0.007	0.030 ± 0.001	0.032 ± 0.002
14	0.015 ± 0.001	0.019 ± 0.002	0.010 ± 0	0.005 ± 0.001	0.012 ± 0.002	0.010 ± 0.003
21	0.015 ± 0.002	0.018 ± 0.001	0.0015 ± 0.002	0.013 ± 0.003	0.015 ± 0.002	0.014 ± 0.001

Table 3. Nitrate content recorded

DAY	CONTROL			FISH TREATED WITH PROBIOTICS (10mg/l)		
	LOW	MEDIUM	HIGH	LOW	MEDIUM	HIGH
0	2.2 ± 0.78	2.2 ± 0.78	2.2 ± 0.78	2.2 ± 0.78	2.2 ± 0.78	2.2 ± 0.78
7	1.6 ± 0	3.3 ± 0	5.8 ± 0.83	5.8 ± 0.83	5.8 ± 0.83	5.0 ± 0
14	5.5 ± 0.50	4.5 ± 0.50	5.5 ± 0.50	6.5 ± 0.50	7.0 ± 0.10	7.5 ± 0.50
21	6.8 ± 0.62	5.6 ± 0.62	6.2 ± 0	7.5 ± 0	8.1 ± 0.62	8.1 ± 0.62

Table 4. Dissolved oxygen recorded

DAY	CONTROL			FISH TREATED WITH PROBIOTICS (10mg/l)		
	LOW	MEDIUM	HIGH	LOW	MEDIUM	HIGH
0	6.3 ± 0.30	6.3 ± 0.30	6.3 ± 0.30	6.3 ± 0.30	6.3 ± 0.30	6.3 ± 0.30
7	2.8 ± 0.19	0.9 ± 0.07	0.7 ± 0.07	2.5 ± 0.07	2.3 ± 0.15	1.7 ± 0.07
14	3.3 ± 0.13	1.2 ± 0.52	0.02 ± 0.01	4.64 ± 0.12	1.1 ± 0.72	0.02 ± 0.007
21	1.8 ± 0.004	1.0 ± 0.009	2.6 ± 0.09	2.1 ± 0.004	1.0 ± 0.004	2.0 ± 0.11

Table 5. Oxygen consumption rates

DAY	CONTROL			FISH TREATED WITH PROBIOTICS (10mg/l)		
	LOW	MEDIUM	HIGH	LOW	MEDIUM	HIGH
0	2.2 ± 0.22	2.2 ± 0.22	2.2 ± 0.22	2.2 ± 0.22	2.2 ± 0.22	2.2 ± 0.22
7	3.6 ± 0.07	3.3 ± 0.13	3.6 ± 0.06	2.6 ± 0.07	2.7 ± 0.07	2.1 ± 0.07
14	1.2 ± 0.17	0.4 ± 0.20	0.4 ± 0.07	0.8 ± 0.26	0.4 ± 0.26	0.3 ± 0.16
21	1.3 ± 0.15	1.2 ± 0.17	0.2 ± 0.09	1.6 ± 0.09	0.7 ± 0.13	0.4 ± 0.06

DAY	CONTROL			FISH TREATED WITH PROBIOTICS (10mg/l)		
	LOW	MEDIUM	HIGH	LOW	MEDIUM	HIGH
0	0.008 ± 0	0.008 ± 0	0.008 ± 0	0.008 ± 0	0.008 ± 0	0.008 ± 0
7	0.055 ± 0.003	0.033 ± 0.004	0.011 ± 0.004	0.025 ± 0.003	0.029 ± 0	0.040 ± 0.003
14	0.085 ± 0.002	0.051 ± 0.001	0.029 ± 0.004	0.010 ± 0.002	0.020 ± 0.001	0.018 ± 0.002
21	0.039 ± 0.005	0.017 ± 0.003	0.010 ± 0.003	0.007 ± 0	0.014 ± 0	0.007 ± 0

Fig. 3. Ammonia excretion rates

4. DISCUSSION

As new findings emerge, several definitions of probiotics have been proposed. Few scientists have given a precise definition of probiotics, which are still widely referred to as live microbial food additives that have a beneficial effect on the host animal by improving its intestinal balance. As noted above, interactions between microbiota, including probiotics, and the host are not limited to the gut. Probiotic bacteria can also be active in the gills or skin of the host, as well as in the environment around it. The intense interaction between the culture medium and the host in aquaculture means that many probiotics are obtained from the culture medium. The knowledge about probiotics has increased, it is now known that these microorganisms have an antimicrobial effect, changing the intestinal microbiota, secrete antibacterial substances (bacteriocins and organic acids), compete with pathogens to prevent their attachment in the intestine, compete for nutrients needed by the body a pathogen by survival and production of antitoxins. Probiotics are also able to modulate the immune system, regulate the body's allergic reactions and reduce the spread of cancer in mammals. Therefore, at a certain concentration and viability, probiotics have a beneficial effect on the health of the host. In fact, terms like "friendly bacteria", "friendly" or "healthy" are often used to describe probiotics. For many years, research has focused on microorganisms characteristic of the gut microbiota, and the term "probiotic" has been largely limited to Gram-positive lactic acid bacteria, particularly representing the genera Bifido, Lactobacillus, and Streptococcus. Unlike terrestrial animals, the microbiota of the digestive tract of aquatic species is particularly dependent on the external environment due to the flow of water through the digestive tract. Most bacteria are therefore transient in the intestine due to constant consumption of water and food and the

microorganisms they contain. Although potentially pathogenic bacteria such as Salmonella, Listeria and Escherichia coli have been reported in the gastrointestinal tract (GIT) of aquatic animals, probiotic bacteria and other microorganisms have also been identified. These include Gram-positive bacteria such as Bacillus, Carno-bacterium, Enterococcus and several species of Lactobacillus; gram-negative facultative anaerobes such as Vibrio and Pseudomonas, and certain fungi, yeasts and algae of the genera Debaryomyces, Saccharomyces and Tetraselmis. With increasing interest in probiotics in aquaculture, Moriarty proposed expanding their definition to include “live microbial supplements that benefit the health of hydrobionts and thus increase productivity. Based on a review of previously published articles, too many works have focused on GIT bacteria or GIT (Gastro Intestinal Tract) bacteria, also known as gut bacteria, to show a huge benefit to aquatic organisms. Therefore, this study was conducted to test the unique role of soil probiotic bacteria and their effects on Oreochromis (Tilapia) fish. Based on current results, this study contributes to improves the quality of fish fed probiotics, such as better fish growth and survival, improved digestion, stronger immune response to pathogenic microorganisms and resistance to stress, reduced dissolved and particulate carbon, lower ammonia and nitrite levels in ponds of fish, and others. It plays an important role in the conversion of nitrite to nitrate in ponds, reducing pollutant levels in fish ponds, increasing the life of forage tilapia fish, and effectively releasing the commercialization of tilapia fish fed a probiotic diet. This study showed that probiotic soil bacteria can improve fish metabolic performance, thereby increasing fish biomass, disease susceptibility, and improving fish digestive processes and improving water quality.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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